review

Haploidentical Stem Cell Transplantation

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The feasibility of stem cell transplantation across the major histocompatibility (MHC) barrier—as in haploidentical stem cell transplantation (SCT)—has been proved for some time in several studies. The main limitations include a higher graft failure rate, delayed immune reconstitution after transplantation with high rates of life-threatening infections, a higher incidence of post-transplant lymphoproliferative disease (PTLPD), and severe acute and chronic graft-versus-host disease (GVHD). In an attempt to reduce the transplant-related morbidity/mortality, several techniques had been evaluated involving conditioning regimen intensity, graft engineering, post-transplant cellular therapy and immunosuppression. This review will describe the current situation. It will also discuss initiatives and strategies to overcome the limitations associated with transplant across the MHC barrier.

Bone marrow transplantation, through a variety of cellular, humoral and cytokine pathways, is a potentially curative therapy for different hematologic disorders. Although the majority of allogeneic marrow transplantations have been performed using either HLA-identical sibling or closely HLA-matched (ie, one-antigen–mismatched) family donor, alternative donor sources such as unrelated or more highly HLA-disparate family donors have emerged as alternative options under certain conditions.[1]

The probability of finding HLA-matched sibling donors is not high and is always decreasing due to the modern trend towards lower family size. The search for matched unrelated donors is time consuming, and might interfere with the optimum timely management of the patients. Again, the utilization of cord blood as a source of stem cells suffers from several limitations, including the low stem cell content as compared to the requirement of an adult patient, late engraftment after transplantation and limitations in post-transplant cellular therapy (boost stem cell dose and donor lymphocyte infusion).[2]

Stem cell transplantation across the major histocompatibility complex (MHC) barrier—as in haploidentical SCT—has been tried in the past, with several limitations including higher graft failure rate, delayed immune reconstitution after transplantation with high rates of life-threatening infections, a higher incidence of post-transplant lymphoproliferative disease (PTLD), and severe acute and chronic graft-versus-host disease (GVHD).[3,4]

In this article, we will try to find answers from the published literature to the questions that might help to further improve the outcome of haploidentical transplantation. These questions are:

1. What is the best conditioning regimen?
2. What is the optimum graft composition?
3. What is the best graft engineering method?
4. What is the best GVHD prophylaxis in this particular setup?

Conditioning regimen in haploidentical stem cell therapy

Myeloablative vs reduced intensity conditioning

Both a myeloablative and reduced intensity conditioning regimen have been used in haploidentical transplantation. In most of the studies using myeloablative conditioning, primary engraftment was obtained in the vast majority of patients with a relatively low incidence of GVHD. However, non-relapse mortality was relatively higher. Opportunistic infection was the main cause of non-relapse mortality (NRM) in most of the

studies, reflecting delayed immune reconstitution after myeloablative conditioning and vigorous T cell depletion.[5-12]

Table 1 shows the data from several clinical trials employing different myeloablative conditioning regimens.

Other reports have evaluated reduced intensity conditioning regimen before infusion of haploidentical stem cells, based on the following principles:

1. The less transplant-related toxicity and early mortality of this technique,
2. The potentially lower incidence of GVHD, yet preserving a potent graft versus malignancy (GVM) effect following donor lymphocyte infusion (DLI).

The engraftment rates in these studies were comparable to that after a myeloablative regimen. Reduced intensity conditioning regimens were not associated with lethal viral infection in most reports which could be explained by the rapid immune reconstitution, fast recovery of thymopoiesis, and by a rapid recovery of the T cell receptor repertoire. This is translated into less transplant-related mortality. However, there is a higher incidence of relapse related mortality.[13-19]

Table 2 shows some of the reduced intensity conditioning haploidentical transplant studies and their results.

Anti-thymocyte globulin (ATG)-based conditioning regimen

The advantage of including ATG in conditioning was obvious from several studies. First, ATG has a relatively prolonged half-life in vivo and can be detected even 30 days or longer after its administration.[20] Thus, it was found to potently delete T lymphocytes for a long period in vivo, preventing GVHD with no increase in incidence of relapse.[21]

Second, ATG included in conditioning results in a faster donor chimerism after HCT, especially for transplantations from alternative donors.[22]

However, the use of ATG in the conditioning regimen is not priceless. It can damage hematopoietic stem cells and retard immune reconstitution for several months after BMT.[23] The use of anti-thymocyte globulin (ATG) for ex vivo T-cell depletion was not impressive. In a clinical trial conducted at the Massachusetts General Hospital, cyclophosphamide, equine anti-thymocyte globulin (ATG) for ex vivo T-cell depletion and pre-transplant thymic radiation were given before haploidentical transplant. Because of a high incidence of acute GVHD in the initial cohort of patients, MEDI-507 (a monoclonal anti-CD2 antibody) was substituted

Table 1. Myeloablative conditioning regimen for haploidentical transplant.

Centre	Disease	Patients (n)	Conditioning	GVHD prophylaxis	NRM (%)	Survival	Reference
University of South Carolina	AML/ALL	201	TBI/VP-16/CY/ ARA-C/ATG, CYA	Partial TCD, CSA, ATG/MP	51	DFS: 18% OS: 19% @ 5 years	Mehta J,et al 2004[5]
Japan (multicenter)	Leukemia, MDS	135	BU/CY/ARA-C/ MECCNU/ATG 22 64%/71% @ 2 years	CSA/MTX/MMF	22	DFS: 64% OS: 71% @ 2 years	Kato S et al , 2000[6]
Teubingen 1	HM, NMD	63	TBI or BU + CY/TT ± FLU	TCD PBSC	29	DFS: 48% @ 3 years (ALL, NHL in CR)	Lang P et al 2004[7]
Emory	HM	28	ATG based myeloablative	TCD	64	OS: 7%	Walker EK et al, 2004[8]
Royal Marsden	AML/ALL	35	TBI/CY or TBI/MEL	CSA±MTX	34	OS: 31% @ 6-36 months	Powles RL et al 1983[9]
Perugia	AML/ALL Refractory/ CR1/ CR2	255	TBI-TT- FLU-ATG	T cell depletion	41	DFS ALL: 25% DFS AML: 40% @3 years	Aversa F 2008[10]
Beijing	HM	171	Ara C- Bu12- Cy-ATG- Me CCNU	CSA, MMF, MTX	22.8	OS: 64.9% @ 2 years	Huang XJ et al 2006[11]

AML: acute myeloid leukemia, ALL: acute lymphoblastic leukemia, TBI: total body irradiation, Cy: cyclophosphamide, VP-16: etoposide, ATG: anti-thymocyte globulin., TCD: T-cell depletion, CSA: cyclosporine A, MP: methyl prednisolone, MDS: myelodysplastic syndrome, BU: busulfan, MTX: methotrexate, MMF: mycophenolate mofetil, HM: hematologic malignancy, NMD: non-malignant disease, TT: thioptepa., Flu: fludarabine, PBSC: peripheral blood stem cell, Mel: melphalan, GvHD: graft versus host disease.

Table 2. Reduced intensity conditioning regimen for haploidentical transplant.

Center	Disease	Patients (n)	Conditioning	GVHD prophylaxis	NRM (%)	Survival	Reference
Teubingen 2	Refractory HM, SAA	38	Flu, thiotepa, melph, and the anti-CD3	MMF for patients who received >25 000/kg T cells	2.6	DFS (good risk): 70% DFS (bad risk): 20% @ 2 years	Handgretinger R et al 2007[13]
St. Jude Children's Research Hospital	Refractory HM	22	Flu, thiotepa, melph, and the anti-CD3	TCD PBSC	12	DFS: 36% @18 months	Chen X et al, 2006[14]
Massachusetts General Hospital	Leukemia, lymphoma	12	CY, Anti-CD2 Mab, thymic XRT	CSA (‡35 d)±ex vivo TCD PBSC	25	DFS: 17% OS: 25% @ 2 years	Spitzer TR et al, 2003[15]
Johns Hopkins	Leukemia, MDS	13	TBI/CY/FLU post-BMT CY	CSA, MMF	8	DFS: 38% OS: 46% @ 6 months	O'Donnell PV et al 2002[16]
Japan (multicentre)	Leukemia, NHL	35	Myeloablative (n=24) Non-myeloablative (n=11)	Microchimeric NIMA mismatched donor SCT tacrolimus ± other drugs	31	DFS: 40% OS: 43% @ 20 months	Ichinohe T et al 2004[17]
Dukes University	HM, SAA	49	Flu -Cy-Campath GCSF	MMF±CSA	30.2	OS: 31%for HM 63 %for SAA @ 1 year	Rizzieri DA et al, 2007[18]

HM: hematologic malignancy., SAA: severe aplastic anemia, Flu: fludarabine., Mel: melphalan., MMF: mycophenolate mofetil., TCD: T-cell depletion., GvHD: graft versus host disease, NHL: non Hodgkin's lymphoma, NIMA: non-inherted maternal antigens, G-CSF: granulocyte colony stimulating factor, CSA: cyclosporine A.

for ATG. After several modifications of the regimen to address problems of GVHD and graft rejection, the current conditioning regimen includes cyclophosphamide and fludarabine, MEDI-507 and thymic radiation.[15] This study points to the fact that although engraftment across MHC was feasible using ATG based conditioning, GVHD remained a problem and additional measures should be taken to address this problem.

Alemtuzumab-based conditioning regimen

Alemtuzumab (Campath-1H) is a humanized monoclonal antibody directed against human CD52 that is expressed at a high density on B, T cells and dendritic cells, but not on hematopoietic stem cells.24 The unique pharmacokinetic profile, in which a lympholytic concentration remains for approximately 2 months after transplantation, may contribute to the potent effect against GVHD. Depletion of host dendritic cells, which also express CD52, could be another mechanism to prevent GVHD.[25,26] The prophylactic effect of alemtuzumab against aGVHD might be much stronger than that of ATG.[27]

Kanda et al evaluated the safety of unmanipulated peripheral blood stem-cell transplantation from mismatched unrelated donors using an alemtuzumab-based conditioning. All 12 studied patients achieved early post-transplant neutrophil engraftment with complete donor-type chimerism and a cumulative incidence of grades III to IV acute GVHD of only 9%.[28] Another advantage of alemtuzumab is that it eliminates not only T cells but also B cells, and thus there might be a lower risk of post-transplant lymphoproliferative disorders. However, alemtuzumab-based conditioning is associated with strong immune suppression and a delayed immune reconstitution after transplantation, which might lead to higher incidence of CMV reactivation.[29]

Another possible disadvantage of alemtuzumab is that it might eliminate NK cells, which might be important for a GVM effect.[30]

Koh et al reported that the lympholytic effect of alemtuzumab on NK cells was weaker than that on T cells and the use of alemtuzumab for ex vivo T cell depletion—alemtuzumab "in the bag"—resulted in the 99.8% and 94% depletion of CD4+ and CD8+ T cells, respectively, whereas 30% of NK cells were conserved in the graft.[31]

Alemtuzumab had been reported to increase the incidence of grade II-IV cardiac complications according to the Bearman criteria.[32] It might also increase the incidence of relapse after stem cell therapy.[33,34] Therefore, alemtuzumab might be appropriate for patients with low disease burden, whereas it may be better to avoid alemtuzumab-based conditioning for patients with active and more advanced disease.

Graft processing and composition

Until the last decade, the wide application of haploidentical transplant was limited by the high incidence of GVHD in unmanipulated transplant and graft failure in extensive T-cell depleted transplants. However, the past decade witnessed great progress in graft engineering which together with the development of new conditioning protocols has allowed for better outcomes.[35] The outcome of different depletion techniques depends on the graft composition that can be achieved by this technique. The most important constituents of the graft in this respect is expected to be CD34+ cells, T cells, B cells and NK cells.

Graft processing might be done by several techniques. Positive and/or negative selections of hematopoietic stem cells are the most commonly used. In negative selection, unwanted contaminating cells are eliminated using monoclonal antibodies, which are specifically directed against line-specific antigens. Contaminating cells are eliminated by means of lysis by complement or through physical or immunomagnetic systems.[35]

Positive selection techniques that are based on CD34+ antigen selection are the most commonly used when selecting hematopoietic stem cells for clinical use.[35] Several systems had been used to process the graft using either negative selection, positive selection or a combination of both methods. **Table 3** summarizes these systems.

CD 34+ dose

The use of megadose of CD34+ stem cells to induce engraftment across MHC barrier was suggested by Rachamim et al. They evaluated the effect of purified CD34+ cells on primary cultures of cytotoxic T lymphocytes. This study showed that CD34+ cells can inhibit the development of specific cell-mediated immune responses against their own HLA antigens but not against third party. Also, they noticed that this "veto effect" requires two conditions: direct contact between the effector cells and the CD34+ cells and the optimum CD34+/effector ratio should be 0.5-1.[40,41]

Although the optimal dose of stem cells in the graft varied from one study to another, there is agreement between different studies that a higher stem cell dose is required to overcome the MHC barrier.[42,43] The Tübingen group and others showed that CD34+ cell dose less than 8×10^6/kg might not be sufficient to achieve engraftment across the MHC barrier,[19,44] while the Perugia group observed good engraftment at an average CD34+ cell dose of 10×10^6/kg.[45]

However, the CD34+ cell dose sufficient for engraftment across the MHC barrier seems to depend on several other factors, including the immune suppressive component of the conditioning regimen and the presence of other graft facilitating cells in the harvest. In a second report from the Tübingen group, they used T cell depletion strategy where T and B cells (CD3/CD19) were negatively depleted in the PBSC harvest. They achieved a level of T cell depletion equal to 3.5 to 4 logs using anti-CD3- and anti-CD19-coated microbeads on a CliniMACS device. The collected graft was then infused into adult patients following reduced dose conditioning. In contrast to the CD34+ positive selection strategy pioneered by the Perugia group, CD3/CD19-depleted grafts harvested using this strategy not only contain CD34+ stem cells, but also CD34– progenitors, natural killer, dendritic and graft-facilitating cells. In this study, the presence of other graft facilitating cells allowed better engraftment even with lower average CD 34+ cell dose (8.6×10^6 vs 10×10^6/kg). This study demonstrated the interactive relationship among the conditioning regimen, graft composition and transplant outcome.[46]

T cell

The level of T cell depletion from the graft that would

Table 3. Some of the commonly used ex vivo graft manipulation techniques.

Method	Mode of processing	Name of the system	Reference
SBA agglutination and E-rosetting	Negative selection	-	Aversa F et al 1994[36]
E-rosetting + immunoadsorption	Negative and positive selection	Ceprate system	Aversa F et al 1998[37]
Immunomagnetic and anti-CD4/CD8 and CD19/CD22	Positive selection and double negative	Isolex 300i system	Stainer CJ et al , 1998[38]
Immunomagnetic	Positive selection	CliniMACS system	Slaper-Cortenbach IC et al 1999[39]

SBA: soya bean antigen.

allow engraftment and, early immune reconstitution without increased incidence of GVHD, was evaluated in several studies.[47-49] A median of 3 to 4 logs T cell depletion was not enough to prevent GVHD in a matched transplant.[47] However, more than 4 logs T cell depletion allowed good engraftment with no GVHD in the same study and in another 43 patients who underwent haploidentical transplantation.[38]

The Acute Leukemia Working Party (ALWP) of the European Blood and Marrow Transplant (EBMT) Group conducted a survey of fully haploidentical hematopoietic stem cell transplantation in 266 adults with high-risk acute leukemia. They reported that an ATG-based conditioning regimen followed by ex vivo T-cell depleted graft to a mean of 1×10^4 CD3+cells/kg (range, 0-6.4) prevented GVHD without any post-transplant immune suppression.[50]

The Perugia group reported the outcome of 255 acute leukemia patients after haploidentical stem cell therapy. The median T cell count was 2×10^4 cell/kg. After a fludarabine-based conditioning regimen, they achieved a 95% engraftment rate, which rose to 98% after a second haplotransplant with further immunosuppression with fludarabine, cyclophosphamide and ATG. GVHD was prevented without any post-transplant immunosuppression, confirming that a threshold dose of 2×10^4 CD3+ cell/kg prevents severe GVHD as long as it is associated with ATG in the conditioning regimen. Disease relapse as well as infectious complications remained high after such an approach.[10]

However, in the Aversa et al study, relapse rates were lower than expected in patients who had unfavorable prognostic features at transplant, and where 18% and 30%, respectively, of AML and ALL patients transplanted in any CR relapsed. Relapse rate was significantly lower after transplantation from NK alloreactive donors (3% vs 47%). Also, 41% of patients died of non-relapse causes. Most deaths were caused by infections, the majority of which were CMV and Aspergillus. In this study, the cumulative incidence of non-relapse mortality was related to disease stage at transplant and was significantly higher in patients who were transplanted in relapse (58% vs. 36%).[37] However, the 'megadose' approach, the use of a reduced intensity conditioning regimen with less damage to the thymus, rapid preemptive antiviral therapy, especially for adenovirus (ADV), and the use of ADV-specific T cell infusion for some patients were all shown to reduce the incidence of non-relapse mortality following haploidentical transplant at the Tübingen study.[19]

Table 4. Different graft composition in different studies and its relation to the outcome.

Center	No	Disease	Conditioning	TCD	Median CD34+ dose 10^6 /kg	Median CD3+ dose 10^4/kg	Engraftment % of patients	Post transplant IS	GVHD grade ≥II %	Relapse	NRM %	Survival %
Perugia[10]	255	AML/ ALL Refractory/ CR1/ CR2	TBI-TT- FLU-ATG	EX VIVO (CliniMacs)	12.8	1	95	No	0	18% in AML, 30 % in ALL	41	DFS 25 % in ALL 40% in AML- 3y
Beijing[11]	171	Hemat. malignancy	Ara C- Bu12-Cy-ATG-Me CCNU	In Vivo	1.8	22000	100	CSA, MMF, MTX	55 aGVHD 73.6 cGVHD	18.7	22.8	64.9
Dukes University[18]	49	Hemat. Malignancy CR1-CR2-PD /marrow failure	Flu –Cy-Campath GCSF	InVivo	13.6	46000	94	MMF±CSA	8	49	30.2	OS 31 63 for SAA -1 y
Osaka University[72]	26	Hemat. Malignancy Advanced stage	Flu-Bu4- ATG	In Vivo	6.5	25400	96	Fk506- mPL	14 /2 antigen mm 27 / 3 antigen mm 80 cGVHD	NA	NA	
University of s. Carolina[5]	201	AML/ALL	TBI- Cy-VP16-Ara C-ATG	Ex Vivo mAB e.g. OKT3	Median total dose of 100	5	98	mPL- CSA-ATG	13 aGVHD 15 CGVHD	31	51	18 DFS 19 OS @5 y

TCD: T-cell depletion, IS: immune suppression, GvHD: graft versus host disease, NRM: non-relapse mortality, AML: acute myeloid leukemia. , ALL: acute lymphoblastic leukemia., TBI: total body irradiation., TT: thiotepa., Flu: fludarabine., ATG: anti-thymocyte globulin., Ara-C: aracytidine., Cy: cyclophosphamide., ATG: anti-thymocyte globulin., CSA: cyclosporine A., MMF: mycophenolate mofetil., MTX: methotrexate., PD: progressive disease, Cy: cyclophosphamide, mPL: methyl prednisolone, mm: mismatch, TBI: total body irradiation, mAb: monoclonal antibodies.

B cells

Table 5. Different approaches for GVHD prophylaxis in haploidentical SCT.

Center	Patients (n)	Conditioning	GVHD prophylaxis			NRM (%)	aGVHD	cGVHD	Survival	Reference
			Immune suppressant	ATG	Ex vivo TCD					
University of South Carolina	201	Myeloablative conditioning	CSA, MP	Yes	Partial TCD,	51	13%	15%	DFS: 18% OS: 19% @ 5 years	Mehta J et al 2004[5]
Japan (multicentre)	135		CSA/MTX/MMF	Yes	No	22	40%	55%	DFS: 64% OS: 71% @ 2 years	Lu DP et al 2006[72]
Teubingen	63		No	No	Yes	29	7%	13%	DFS: 48% @ 3 years (ALL, NHL in CR	Lang P et al 2004[7]
Emory	28		-	Yes	Yes	64	NS	NS	OS: 7%	Waller EK et al 2004[8]
Royal Marsden	164		CSA ± MTX	-	No	51%	NS	NS	OS: 31% @ 6-36 months	Singhal S et al 2003[73]
Perugia	255		T cell depletion	Yes	Yes	41	-	-	DFS ALL: 25% DFS AML: 40% @3 years	Aversal F 2008[10]
Beijing	171		CSA, MMF, MTX	Yes	No	22.8	55%	46.9%	OS: 64.9% @ 2 years	Huang XJ et al 2006[11]
Tübingen 2	28	RIC+OKT-3	-	No	Yes	39% @ 2 years	54%	18%	OS 31% @ 2 years	Federmann B et al l 2011[46]
St. Jude Children's Research Hospital	22	RIC=22 Myeloa-blative= 12	MMF± CSA	No	Yes	-	36%	22%	25% @ 2 years	Chen X et al 2006[14]
Massachusetts General Hospital	12	RIC+ anti CD2	CSA	No	Yes=4 No=8	na	-	-	-	Spitzer TR et al 2003[15]
Japan multicentre study	35	Myeloa-blative=24 RIC=11	Tacrolimus ± methotrexate ± steroids ± MMF	No	No	11\35	56%	83%	OS=43% DFS=40%	Ichinohe T et al 2004[17]
Dukes University	49	RIC+ campath-H1	MMF±CSA	No	No	15\49	16%	14%	DFSS=31% OS=43% @ 1 year	Rizzieri DA et al 2007[18]

SCT: stem cell transplant, GvHD: graft versus host disease, ATG: anti-thymocyte globulin., NRM: non relapse mortality, CSA: cyclosporine A., MP: methyl prednisolone., TCD: T-cell depletion., MMF: mycophenolate mofetil., MTX: methotrexate., AML: acute myeloid leukemia. , ALL: acute lymphoblastic leukemia., Non-hodgin's lymphoma., RIC: reduced intensity conditioning

Extensive T-cell depletion without comparable B-cell depletion was associated with a significant increase in the incidence of Epstein Barr virus-induced lmphoproliferative disorder (EBV-LPD), especially with the use of ex vivo graft engineering.[51-54] Also T-cell depletion together with use of ATG and a high CD34+ cell count in the graft were the most important factors influencing post-transplant EBV reactivation.[55,56]

Several investigators had suggested that the ratio of T and B cells in the graft is important for EBV surveillance. Meijer et al suggested that a T/B cell ratio of 2.5 is sufficient to prevent EBV-LPD.[51] However, the optimal T/B cell ratio is currently not known and is dependent on several factors, such as use and dosage of ATG.[51,57]

Cavazzana et al showed that none of the patients who had received transplants from a partially matched-related donor (PMRD) developed EBV-LPD when ex vivo T and B cell depletion was performed, whereas 7 of 19 historical controls developed EBV-LPD when only T cell depletion was carried out.[58] In this study, fewer than a median of 0-5×10^6 B cells were infused to recipients. This data was confirmed in the study of Aversa et al, where B-cell depletion was 2.3 logs and the infused B-cell dose was 4.09-2.53×10^5/kg. The incidence of EBV-related lymphoproliferative disorders was less than 3 %.[45]

NK cell

After extensive T cell depletion, the NK cell becomes the main effecter cell in the graft versus leukemia (GVL) reaction. HLA class I disparities driving NK cell alloreactions in the GVH direction mediate strong GVL effects, produce higher engraftment rates and do not cause GVHD. NK cell alloreactivity may be a unique therapeutic tool for tolerance induction and clearance of leukemia in hematopoietic transplantation.[59-61] Bachanova et al reported that IL-2 activated autologous NK cells can induce, but not maintain durable remissions in lymphoma patients.[62]

The relationship between the graft content of NK cells and the effectiveness and safety of haploidentical transplantation was not adequately studied. However, in a multivariate analysis by the Korean study of 61 myeloabative conditioning allo-PBSCT from matched sibling, there was strong correlation between higher dose of NK cells (≥5×10^7/kg) and a lower incidence of non-relapse mortality (NRM), GVHD and infectious events.[61]

In the Tübingen study, the graft contained an average of 137×10^6/kg CD56+ NK cells (range 9–550). The rate of engraftment was 83%. Acute GVHD grades 2-4 were seen in 27% of patients. GVHD prophylaxis consisted of CD3 depletion and post-transplant mycophenolate mofetil starting from day 1 in those patients who received greater than 25 000/kg T cells with the graft. NRM of 2.6% with EFS of 70% for the good risk group and 20% for the relapsed/refractory group were observed.[19]

Rather than the dose of NK cells in the graft, the speed of NK cell recovery looks to be more important in this regard. Early NK cell recovery as occurring in the setup of reduced intensity conditioning would be expected to have positive effect on GVL reaction, antiviral immunity and suppression of the GVHD.[18,19,46,63-65]

The data from these studies suggest that the higher graft content of alloreactive NK cells would be expected to ablate leukemic cells and recipient T cells, permitting the use of reduced intensity conditioning regimen. Also, it can ablate the recipient dendritic cells (DCs) which trigger GVHD, thus protecting from GVHD while permitting a higher T cell content in the graft. In other words, the graft composition and the relative content of each of the cellular components in the graft have direct implication on the outcome of haploidentical SCT. **Table 4** shows different graft composition in different studies and its relation to the outcome.

Haploidentical stem cell therapy and GVHD

Being a major problem in haploidentical SCT, GVHD prophylaxis has attracted the attention of several study groups. Some of these studies used immunosuppressive agents while others depended on variable degrees of T cell depletion or a combination of both. In spite of that, 30% to 70% of haplotransplant recipients suffer from GVHD.[2,66,67,68]

The Italian studies focused on myeloabaltive conditioning followed by infusion of a megadose of CD34+ cells with extensive T-cell depletion and no post-transplant immunosuppressive agents. Although they reported a lower incidence of GVHD, the procedure was associated with significant TRM of 35 to 40 %, mainly due to delayed immune recovery and infections.[12,35,37,45,69]

The German group tried to overcome this problem by reducing the intensity of conditioning and targeting a higher dose of more than 10×10^6 CD34+ cells/kg body weight with extensive T-cell depletion. Although engraftment was rapid at a median 15 days, T- and B-cell reconstitution was delayed, whereas NK cell reconstitution occured early and fast. aGVHD occurred in 54% of patients, while 18% developed cGVHD.[13,19,44,46]

Cellular therapy, which theoretically not only reduces GVHD, but also augments the graft-versus-leuke-

mia (GVL) effect, had been studied in this field. An example of this cellular therapy is infusion of alloreactive NK.[62,69] Pre- and post-transplant infusion of alloreactive NK cells either in preparation for the coming graft or similar to donor lymphocyte infusion for relapsed cases has been suggested to have the same positive effect on engraftment and graft versus host disease.[61,62,69,70]

De Angelis et al assessed the role of different NK subsets in exerting GVL effects in recipients of haploidentical transplants. They reported that CD3–/CD56– cells expressed NK cell-associated molecules, such as CD16, NKp46, NKp30, CD244 (2B4), CD161, and killer cell immunoglobulin-like receptors. CD3–/CD56– cells further exhibited the classical functional characteristics of NK cells: cytolysis of target cells lacking HLA class I, antibody-dependent cellular cytotoxicity and cytokine production. These results demonstrate that CD56– NK cells are functional, recognize missing self and, like their CD56+ counterparts, may contribute to GVL reactions.[69] Bachanova et al also reported the beneficial effect of the CD56- subset as anti-tumor cells without increasing GVHD incidence.[62] However, further studies are needed to completely establish the technique of donor NK cell collection and infusion.

Another example of cellular therapy is coinfusion of mesenchymal stromal cells (MSCs). MSCs were suggested to have the ability to enhance engraftment and reduce GVHD through preventing T-cell activation and proliferation.[71] Liu et al reported in an open-label, randomized phase 2 clinical study to assess the outcome of MSC coinfusion during haploidentical hematopoietic stem cell transplantation in a total of 55 patients. No immediate or long-term toxic side effects related to MSC infusion were noted. It also helped early engraftment and platelet recovery. There was a trend towards a lower incidence of cGVHD, but a higher incidence of aGVHD in the group who received (MSCs).[71] **Table 5** shows the outcome of some of the approaches to reduce GVHD in haploidentical SCT.

In conclusion, although haploidentical stem cell transplantation is a technically challenging procedure, it carries a high risk of complications and transplant-related morbidity and mortality, yet it remains an option for those who require SCT, but lack a suitable HLA-matched graft either from a related or unrelated donor. The level of immunosuppression that is required to induce engraftment across MHC is feasible whether through conditioning regimen, graft engineering or both. Post-transplant complications, mainly GVHD, delayed engraftment and infectious complications remain considerations. Ongoing research, especially the use of adoptive immunotherapy, chemoprophylaxis against relevant infectious agents and better graft engineering techniques, might bring haploidentical stem cell therapy more into regular practice.

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